Supporting information for:

Mechanistic Investigation of the Androgen Receptor DNA Binding Domain Inhibitor Pyrvinium

Sumanta K. Pal[1], Ben Yi Tew[1], Minyoung Lim[1], Brittany Stankavich[2], Miaoling He[1], Miles Pufall[2], Weidong Hu[3], Yuan Chen[3], Jeremy O Jones[1*]

[1]Department of Medical Oncology City of Hope, [2]Department of Biochemistry, University of Iowa. [3]Department of Molecular Medicine, City of Hope

Corresponding Author and to whom reprint requests should be made:

Jeremy Jones

1500 E Duarte Rd

Duarte, CA 91010

Phone: 626-256-4673 ext 80270

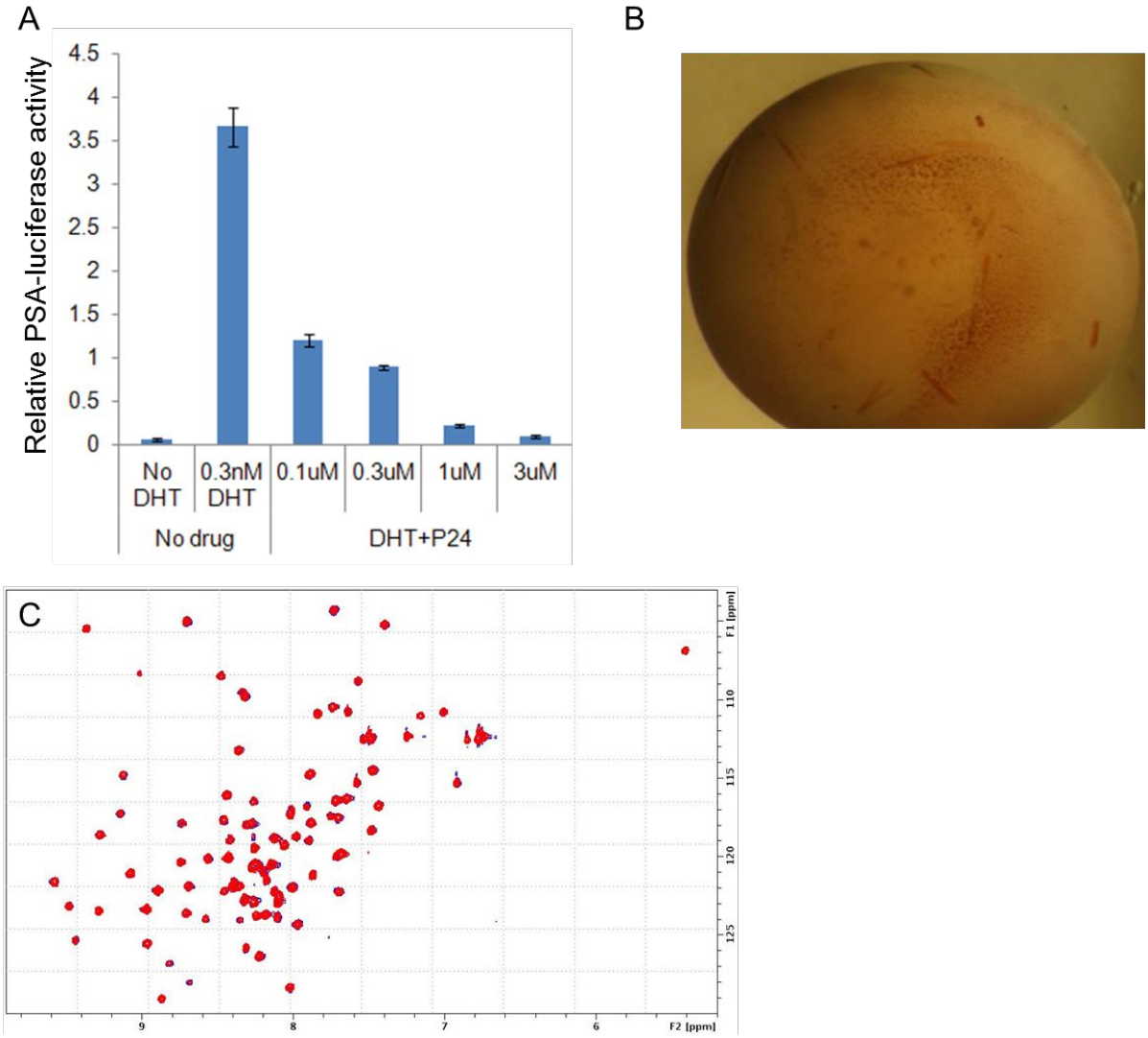
Fax: 626-471-3902

Email: jjones@coh.org

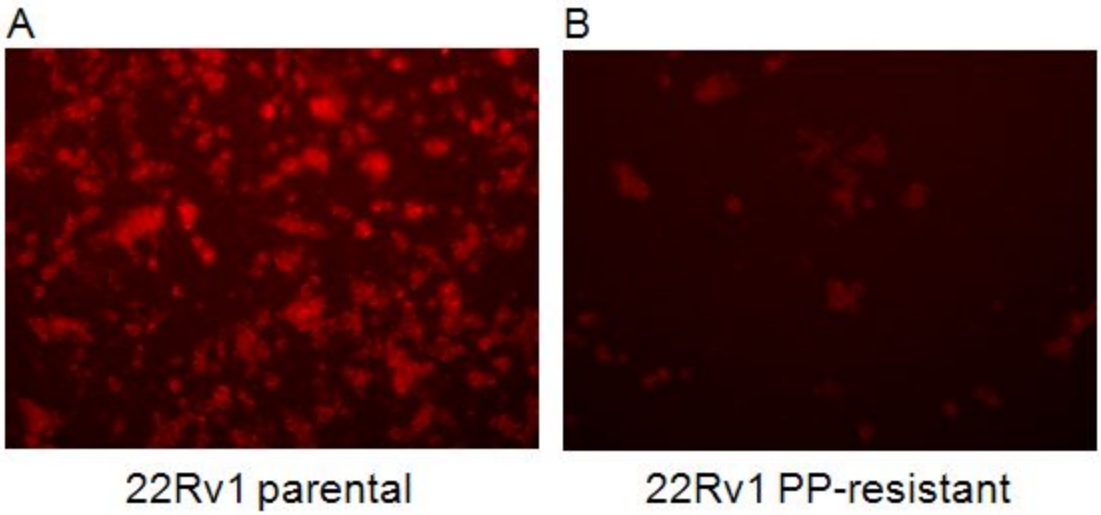
Supplementary Methods:

Crystallization efforts: Crystallization studies were performed by SARomics Biostructures, Sweden. Initially the commercial crystallisations screens JCSG+ and PACT (Molecular Dimensions) were setup for all 6 complexes. Drop ratios of 200 nl:200 nl were used for the protein-DNA complex:reservoir solution, in Rigaku 3-well plates. The reservoir volume for all wells was 45 µl. Prior to the screen a pre-crystallization test was done to determine whether the concentration of the complex was appropriate. The concentration used for initial screening ranged from 10-12mg/ml for all the complexes. The following crystallization screens were used. All screens were set up using a mosquito crystallization robot (TTP Labtech). After setup, plates were put in a Minstrel HT UV plate hotel (Rigaku) for imaging and storage. The incubation temperature was 20C and the imaging interval was 0, 1, 3, 7, 14, 21, 30, 60, 90 and 120 days. The initial screening effort produced 16 hits. After five rounds of optimization, large enough red crystals were grown but diffracted poorly to about 15Å when tested at beamline I911-3 at MAX II synchrotron, Lund, Sweden.

Drug resistant cell line creation: 22Rv1, LNCaP, and LAPC4 cells were cultured in increasing concentrations of PP over a period of several weeks. Eventually, a 22Rv1 cell line was established that could be routinely passaged in 100nM PP. High concentration (100nM) PP exposure and recovery was also attempted without success in any cell line.



Supplementary Figure 1: (A) LNCaP cells were transfected with PSA-luciferase and SV40-renilla luciferase control reporter plasmids. Following overnight treatment with the indicated drugs, luciferase activity was quantified in quadruplicate samples. (B) Crystals of purified AR DBD protein, ADR3 DNA oligo, and P24 were grown, but they diffracted poorly. (C) Overlay of the spectra of AR-DBD in the absence (blue) and presence (red) of P24 showing no difference of the two spectra.



Supplementary Figure 2: 22Rv1 parental cells (A) or PP-resistant cells (B) were treated with 30nM PP overnight. The following day, cells were fixed and fluorescently imaged. PP, which fluoresces in the red range, was diminished in the resistant cells, suggesting expression of a drug exporter.